Past, present and future; the strength of plant-based dairy substitutes ~~and derivative products~~ based on gluten-free raw materials

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Abstract

Plant-based foods are gaining popularity and the market is developing fast. This trend is based on several factors, like the change of lifestyle, interest in alternative diets, and the increasing awareness about sustainable production of food and especially proteins. Plant-based dairy substitutes can serve as an option to traditional food products, meeting many of these interests. However, the market is in its infancy and needs to progress. Trends show, that the market will change from being focused on mainly soya, almond and rice-based products, due to their unsustainable farming, and nutritional concerns, like genetic modification and low protein content. The market is likely to shift towards alternative plants to meet consumers' needs and desire for healthy, flavourful and intriguing products. In this regard, the aspect of allergy-free, like gluten-free products gain in importance. Research studies are approaching the nutritional quality of plant-based dairy substitutes, such as improving the protein quality and glycaemic properties. Furthermore, the application of these products or plant proteins as functional ingredients or substitutes for cow's milk in dairy products like cheese and yoghurt are disseminated. However, there is still a need for much more diversified studies in order to overcome stability, textural, nutritional and sensory problems.

Introduction

The interest in alternative foods is increasing. Driven by the need to feed the growing world population, sustainable, and nutritious food sources are becoming an omnipresent concern, for companies and consumers likewise. Although plant-based dairy substitutes (PBDS) have been consumed for centuries as a traditional part of various cultures, a new interest is developing, and the market for such products is expanding rapidly. Traditional products include Spanish Horchata and also Asian soya milk, whereas today the most popular cow's milk alternative is still soya milk besides almond- and rice-based milk substitutes (BMS) (~~Outi Elina~~ Mäkinen, Wanhalinna, Zannini, & Arendt, 2016). The market for plant-based dairy substitutes (PBDSs) is driven by many interests and influenced by different opinions. Nowadays, the majority of consumers are not choosing PBDSs out of necessity, but out of preference (Mintel Group Ltd, 2016). Especially, protein sources are on everyone's minds, since livestock farming is expending a lot of energy and producing greenhouse gases (Day, 2013). However, such concerns are not the only aspects driving the market; an awakening awareness and longing for tradition and culture can be observed. Ray et al. (2016) described it as "Folk to function" and pointed out the treasure of fermentation and heritage that we can find in this tradition, which has been preserved from generation to generation. Therefore, traditional products like Horchata or fermented plant-based foods like tofu, sufu, or other new developed probiotic products are gaining in popularity. There has been an increase in consumer awareness of the rapid environmental deterioration over the past few decades (Min & Galle, 1997). The shift toward plant based diets, as people become aware of their beneficial health implications also improves food sustainability and environmental impact. Furthermore, consumer aloofness regarding allergens is growing. Not only dairy-free but nut-free, and gluten-free products are gaining in market share (Sarah Theodore, 2015). The avoidance of allergens is increasingly seen as part of a healthy lifestyle.

For all these reasons, the market and therefore as well the interest of research is changing and developing quickly. This review summarises the recent achievements and technological, nutritional, and environmental aspects of PBDS. This review aims to emphasise the importance of these products in terms of nutrition and sustainability and to give an overview on the market situation and consumer interest.

Past; traditional plant-based beverages

Plant-based beverages and derived products have been consumed in early civilisations all over the world. Most of them are available just at the local market or, are prepared traditionally at home on a very small scale in order to provide for the family or the small local community (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003). There is a wide range of indigenous plant-based beverages from around the world. For example, many different rice based beverages originate from Asia; Sikhye, based on cooked rice, malt extract and sugar from Korea; Amazake, a sweet, low or non-alcoholic fermented rice drink from Japan. But similar and other beverages derive from different regions around the world: Atole is a Mexican drink, traditionally prepared with maize. Chicha is a term used for any fermented or unfermented beverage consumed in the Andes based on many different grains and fruits. Bushera is a fermented drink made of sorghum or millet from Uganda. Boza is a fermented drink made of wheat, rye, millet or maize originated from Bulgaria, Albania, Turkey and Romania (Blandino et al., 2003). The "tiger-nut milk" Horchata is an unfermented beverage of milky appearance from Spain (Cortés, Esteve, Frígola, & Torregrosa, 2005). Soya based food products date back to the cornerstone of the traditional Asian diet. Today it is the most widely consumed plant-based milk substitute (PBMS). The first commercially available soya milk product was produced in Asia in 1940 and spread to the Western World rapidly (~~Outi Elina~~ Mäkinen et al., 2016). This success is due to much research on the production of milk flavoured soya milk to meet the consumer expectations and the development of technologies for large scale production.

Present of plant-based milk substitutes; current market situation

Consumer demand for cow's milk alternatives has increased as a result of people being intolerant to cow's milk, including lactose intolerance and cow's milk allergy. Soya milk is still the most common milk-substitute. However, 14% of the individuals who suffer from cow's milk allergy also have reactions against soya (Zeiger et al., 1999). Additionally, throughout the Western World concepts of nutritional needs are expanding from survival and hunger satisfaction to using food to promote health and overall state of well-being (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). Consumer interest in health and wellbeing is growing rapidly across the world (Ali, Ali, & Alam, 2015) and has been led by the increase in knowledge in chronic diseases and the many health claims that are now associated with different foods available. Effectively, most people consume PBMSs out of preference nowadays, rather than necessity due to an allergy (Mintel Group Ltd, 2016). PBMSs are often perceived as healthy possibly due to the health claims associated with them e.g. regarding vitamins, fibre, or cholesterol. This is pushing the market, alongside with the negative perceptions people often associated with cow's milk. Negative perceptions of cow's milk include the possibility to contribute to some human diseases along with the high fat content (Dewhurst, Shingfield, Lee, & Scollan, 2006). In 2015, over 130 variants of different PBMSs were available on the European market and worth 1.5 billion U.S.$. (Mintel Group Ltd, 2016). Additionally, fermented food and beverages are in the spotlight of consumer attention, which is broadening the non-dairy market even wider. New products, like fermented yoghurt and cheese alternatives are occurring on the market. However, the scientific attention is still little for these products and they have not received the scientific attention they deserve in the last decades.

Plant-based milk substitutes

PBMSs are water extracts of dissolved and disintegrated plant material. Several processing steps can be applied in the production. However, the general outline of a modern industrial scale process is essentially the same: the plant material is either soaked and wet milled or the raw material is dry milled and the flour is extracted in water afterwards. Often this slurry is filtered or decanted, to remove the grinding waste and insoluble plant material. Standardisations and addition of other ingredients like oil, flavourings, sugar, and stabilizer may be applied afterwards, depending on the desired product. To improve the suspension and microbial stability, homogenisation and pasteurisation or ultra-high temperature (UHT) treatment take place in the end of the process (Diarra, Nong & Jie 2005). Depending on the plant source and or production steps, the solutions are either colloidal suspensions or emulsions. The finalised product resembles cow's milk in appearance. The general manufacturing steps are displayed in Figure 1. Mäkinen et al. (2016) described the production of PBMSs in detail recently therefore it will not be reviewed in this article. This review will focus on related products such as substitutes for yoghurt, cheese and other derived products and their product technologies, with special emphasis on the fermentation process.

Fermentation in plant-based products

Fermentation is one of the oldest forms of food preservation; additionally it improves nutritional quality and enhances the sensory attributes of the resulting products while being natural and economical (Ross, Morgan, & Hill, 2002). Currently, over 5000 different fermented foodstuffs are consumed by humans throughout the world. In 2014 the fermented milk market was worth 46 billion € worldwide, accounting for almost 77% of the fermented food market (Marsh, Hill, Ross, & Cotter, 2014). Many communities around the world produce fermented cereal products, similar to PBMSs, both small and large scale, using different cereals such as rice, wheat, corn or sorghum (Blandino et al., 2003). The microbes present in these products ~~can be quite complex and~~ are often poorly characterised due to the small scale, home preparation of the products using skills and recipes that have been passed down through the generations. Yeasts, bacteria and fungi are involved in the fermentation, solely or in mixed cultures, working parallel or sequential with a changing dominant flora. The commonly used yeasts are species of *Saccharomyces*, which results in alcoholic fermentation (Piškur, Rozpędowska, Polakova, Merico, & Compagno, 2006). These products are not discussed in this review. Likewise, fungi are not frequently used to ferment PBMSs. However, the majority of non-alcoholic fermented products are soured and mainly based on lactic acid fermentations Bacteria ~~species~~ genus of interest for cereal fermentation are *Leuconostoc, Lactobacillus*, *Streptococcus*, *Pediococcus*, *Bifidobacterium* and *Bacillus* (Steinkraus, 1997). During cereal fermentation many different volatile chemicals are emitted out and perceived by the consumer as aromas; these volatile compounds contribute to the overall flavour of the fermented cereal product (Blandino et al., 2003; Peyer, Zannini, & Arendt, 2016). Fermentation has been shown to significantly increase the nutritional quality of cereals. It can also help to enrich the pool of available amino acids, vitamins and minerals, and consequently fermentation increases the overall digestibility and sensory attributes of the food (Ray et al., 2016). Another enzymatic process of huge value is the reduction of oligosaccharides, responsible for digestion problems like flatulence and occurring in beans and vegetables. Due to fermentation with a *Bifidobacterium*, α-galactosidase were released, which minimized the content of oligosaccharides in soya milk (Donkor, Henriksson, Vasiljevic, & Shah, 2007). The same study showed an increase of angiotensin-converting enzyme (ACE) inhibitory activity, which results in an antihypertensive effect. ACE-inhibitory peptides occur in several food proteins, including the storage proteins of cereals (Donkor et al., 2007; Hu et al., 2011).

Different parameters can be observed which influence the growth of microorganism; the composition and processing of the cereal grains, the substrate formulation, the growth capability and productivity of the starter culture. In general, cereals are supporting microbial growth well and even have been proposed as prebiotics, since they are rich in indigestible matter, like dietary fibre, or certain peptides, proteins and lipids to promote the growth of beneficial bacteria (FAO/WHO, 2001). Inulin and a range of oligosaccharides, present in some fruits, vegetables and cereals are getting most of the scientific attention and showed in several studies the ability to support microbial growth (Bernat, Chafer, Chiralt, & Gonzalez-Martinez, 2015; Capriles & Arêas, 2013; Vijaya Kumar, Vijayendra, & Reddy, 2015).

Fermented drinks

Besides aroma compounds and other minor products, ~~Amongst other things~~ acids are produced most notably during the fermentation of food with bacteria. This results in a sour product with a certain aroma profile. Depending on the base, cereal products ~~the product is often~~ can be diluted or blended with juice, aromas and flavours are added or carbonized in order to get a beverage (Kreisz, Arendt, Heibner, & Zarnkov, 2008). Arora et al. (2010) and Sharma et al. (2014) worked on germinated barley and wheat beverages fermented with *Lactobacillus acidophilus*. Both came to the conclusion that a combination of germination and fermentation is a successful way to improve nutritional quality: it ensured significant improvement by~~in~~ reducing the content of sugars while~~,~~ enhancing the levels of thiamine, niacin, lysine, and soluble dietary fibre in the case of the wheat based study (Arora et al., 2010) and in the barley based study it was shown that germination increases the cell count effectively

(Sharma et al., 2014). ~~-~~It can be observed that a lot of research is based on probiotics and functional food in non-dairy as in dairy products. It is not surprising that a lot of products contain oats for the latter reason. Oats are renowned for their beta-glucan content, which has been officially acknowledged by the FDA and allows products to claim a reduced risk of heart diseases (U.S. Food and Drug Administration 2013). Oats showed in several studies to be a suitable substrate for several bacteria. Several studies from Mårtensson are based on oat-based yoghurt like product (Mårtensson, Maite-Duenas-Chascob, et al., 2002; Mårtensson, Öste, & Holst, 2001), which is described in the section pertaining to yoghurt and further information about oats can be found in the section on nutrition. Probiotics are living microorganisms which contribute to the health of the host, when consumed in adequate amounts (FAO/WHO, 2001). Traditionally, dairy products have been the main vehicle of probiotics in the human diet but nowadays, consumer demand is rising for plant-based alternatives. Therefore, synbiotics, the combination of probiotics and prebiotics (FAO/WHO, 2001) are investigated recently. Casarotti et al. (2014) supplemented milk with quinoa flour to study the probiotic activity. ~~Low levels (up to 3%) of quinoa were added to cow's milk and n~~No changes regarding the growth of *Bifidobacterium animalis* ssp. *lactis* or *Lactobacillus acidophilus*~~, which are frequently used in commercial products~~, were observed when low levels (up to 3%) of quinoa were added to cow's milk. Even this neutral effect is a positive outcome, since it shows that highly valuable quinoa can be added to dairy products to improve nutrition, while not affecting the probiotic quality. Furthermore, not only new developed products are investigated: the interest in traditional products and their detailed microbial flora is growing. For example, the traditional Bulgarian beverage Boza was assessed regarding its microbial flora. The isolated *Lactobacillus. plantarum, Candida rugosa* and *Candida lambica* demonstrated probiotic properties, been resistant to bile up to a concentration of 2% (Gotcheva et al., 2002). In 2008, Todorov showed that LAB's isolated from Boza produced bacteriocins, which were active against a number of pathogens, e.g. *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Mridula & Sharma (2015) investigated the utility of sprouted wheat, barley, pearl mille and green gram in combination with oats, and with or without soya milk in a probiotic drink. Each of the sprouted cereals improved the growth of *Lactobacillus acidophilus* with increasing levels of cereals.

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Yoghurt substitutes

Traditionally, *Streptococcus thermophilu~~e~~s* and *Lactobacillus bulgaricus* are commonly used as starter cultures in yoghurt production based on cow's milk. They live symbiotically and grow rapidly during fermentation. Typically, milk is fermented at 42 -43 °C until pH 4.5~~2~~ to 4.2~~3~~ is reached, which corresponds to 1.2 to 1.4% lactic acid. The bacteria reach finally a population of 20 x 10^8 cells/mL (Chandan & Kilara, 2013; Robinson, 2002). Cow's milk yoghurt is a gel based matrix. Gelation is a critical first step in both cheese making and yoghurt manufacture (Lucey, 2002). In the case of yoghurt, cow's milk is normally heated at a pH (6.6) distant to the isoelectric point (pI) of cow's milk proteins, which causes denaturation and formation of soluble aggregates. During the fermentation with LAB's, the pH is decreasing towards the pI (4.6), resulting in gelation as caseins form a continuous network (W. J. Lee & Lucey, 2004). Dairy alternative yoghurts can have structural disadvantages compared to dairy yoghurts made with animal milks, because of the nature of the proteins. Mäkinen et al. (2014) studied the structure of acidified bovine milk, soya- and quinoa-BMSs. Even though all proteins showed gel-like characters with $G' >> G''$, the quinoa sample showed a weaker gel structure compared to soya and bovine sample. This could be due to either stronger intermolecular association or simply less: the used quinoa-BMS contained little protein with 1.26 compared to 3.32 and 2.95 g/100g for bovine milk and soya-BMS respectively. Another study based on oat-BMS showed similar results: chemically acidified samples with glucono-delta-lactone did not increase the viscosity. The authors hypothesised ~~hypostasised~~ that the proteins are not likely to coagulate and that the protein content was too low to show changes in viscosity (Mårtensson, Öste, & Holst, 2000). Further investigation of Mäkinen et al. (2015) exhibited the sensitive nature of proteins to their environment, such as the pH: heat-denaturation of a quinoa protein isolate at high pH (10.5) prior to an acid induced gel formation led to a strong gel, whereas a heat treatment at lower, neutral pH resulted in a weak gel. At pH 10.5 the proteins were highly soluble and heat-aggregation was retarded, due to increased surface charge. During acidification, the soluble aggregates cross-linked and formed a regular and very fine network. In contrast to that, at a lower pH the proteins formed random disulphide bonds and aggregated already during heating, which resulted in a coarse coagulum. Accordingly, recent studies on oat-protein-isolates investigated the characteristics of gelling in detail.

Different levels of glucono-delta-lactone were added to heated oat-protein-isolates at pH 8, which enabled them to produce different gels, with changing gel network structure and strength. A high pH reduction rate and final pH, near the pI resulted in gels with small pores, dense wall microstructure, and superior mechanical strength, comparable to egg white gel (Yang, Wang, & Chen, 2017). In contrast to the study of Mårtensson et al.(2000), Yang et al. (2017) used oat protein isolates, controlled pH, applied heating and adjusted acidification. By taking all these factors into account, protein gels for many applications can be achieved. However, applying this to a food system can be difficult, since the system might be much more complex and altering the conditions might influence other ingredients or is simply to cost and work intensive. Most commercial yoghurt-like products contain thickeners and emulsifiers to alter the firmness but several attempts have been made to improve the products qualities of plant-based yoghurt substitutes, e.g. by the addition of different ingredients like inulin, raffinose and glucose (Donkor et al., 2007), fructose (Buono, Setser, Erickson, & Fung, 1990), or even whey proteins (S. Lee, Mow, & Se, 1990), lactose (Cheng, Thompson, & Brittin, 1990) and evaporated milk (Buono et al., 1990) or pre-treatments such as ultra-high pressure homogenisation (Fernandez-Avila & Trujillo, 2016) or microwave processing (Bhattacharya & Jena, 2007). Many consumers reject these additives in products: the clean label trend is growing and the use of animal ingredients excludes the vegetarian/vegan consumer segment. One potential method to avoid additives is the use of bacteria that produce exopolysaccharide's (EPS). EPS such as dextran, with predominant α-(1$\rightarrow$6) linkages produced by the enzyme dextransucrase could find applications as food hydrocolloids. Certain strains of lactic acid bacteria (LAB) can produce EPS, which can cause a consistency similar to that of dairy yoghurt. Effects of EPS include improved mouth feel, limited syneresis, ropiness, and increased gel firmness based on its ability to bind water and interact with proteins (Hickisch, Beer, Vogel, & Toelstede, 2016). The amount and composition of the EPS produced by the LAB depends on many different factors such as growth of LAB strain, temperature, initial pH, carbon source and the availability of different minerals, vitamins and other medium components (Amari et al., 2013; Grobben, Boels, Sikkema, Smith, & Bont, 2016; Shukla & Goyal, 2011). While the interaction between EPS and milk proteins is extensively studied the interactions of EPS with plant-proteins have rarely been investigated. Soya proteins and EPS were embedded in a network, formed a stable network, and maintained a high apparent viscosity (966.43 mPa s) during 21 days of storage at 4 °C (C. Li et al., 2014). Another study on lupin-based yoghurt alternatives found that EPS yields were comparable to EPS yields reported for cow's milk yoghurt. *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Lactobacillus brevis* were used as starter cultures with glucose as a carbon source, and yielded in apparent viscosities significantly lower than cow's milk yoghurt. At 50 s−1 viscosities of ~350–1180 mPas were measured compared to ~2000–2400 mPas respectively. They stated that EPS was very likely to be incorporated in a network with proteins and other constituents, but no structural characterizations were performed to investigate the nature of the network (Hickisch et al., 2016). Different heat treatments were applied as well, and like mentioned earlier, this is affecting the protein-network itself, but could change the interaction to EPS also. A similar product based on *Lupinus campestris* seeds fermented by *Lactobacillus delbrueckii* ssp. *b~~B~~ulgaricus* yielded a viscosity comparable to dairy yoghurts after 72 h fermentation at 25 °C (Jiménez-Martínez, Hernández-Sánchez, & Dávila-Ortiz, 2003). In addition, fermentation not only provides texture by altering the pH or forming EPS, it is essential for flavour development. This point is discussed in the section about consumer acceptance and sensory in detail.

Cheese

Milk-based cheese is naturally made with the use of enzymes from rennet and LAB for acidification. The kind and degree of fermentation determines characteristic properties, like the rheology, texture, and certainly taste. Depending on this, many different cheese styles can be achieved (Lucey, 2002). Cheese analogues, just like traditional cheese, are emulsions of oil-in-water, wherein proteins function as emulsifiers and provide structure throughout a gel matrix similar to natural cheese (Bachmann, 2001). However, the production of cheese substitutes follows a different regime, due to the different nature of plant-proteins; in cow's milk, casein is changed to para-casein by the rennet, which interacts with calcium-salts and forms a strong, reticulated curd (Chavan & Jana, 2007). Literature dealing with enzymatic coagulation of peanut- and soya-BMS showed generally weak structures and especially hard style cheeses are difficult to obtain (Santos, Resurreccion, & Garcia, 1989). The so-called cheese of Asia, sufu dates back to ancient Chinese history. It is produced by coagulating soya milk, pressing the resulting curds and following fungal and/or bacterial fermentation (Ahmad, Li, Yang, Ning, & Randhawa, 2008). As coagulate salts like calcium sulphate are traditionally used. The general production steps are displayed in Figure 2. The structure is described as tender but brittle. By using other ingredients, or methods, like acidulants, or the application of enzymes, smoother textures can be achieved since the structure relies on the pore sizes and other microscopic properties of the matrix (Berk, 1992), similar to the system of yoghurt. On the western market, most of the cheese analogues contain dairy ingredients like casein, or milk fat, and are supplying the low value market with cheap products, the most abundant of which being a mozzarella analogue for pizza (Bachmann, 2001). On the other hand, the exclusion of dairy ingredients and inclusion of high value plant-based ingredients serves the market with functional, healthy and diversified food products. Most of the products available on the market consists of partly hydrogenated vegetable oils, plant proteins, stabilizers, emulsifiers, emulsifying salts, acidulants, salt, colouring, flavouring, preservatives, and water, without using starter cultures and enzymes. The ingredients are blended together and most of the time the products are non-matured (Bachmann, 2001). Plant-based cheese-like products attain little scientific attention and most of the studies only partially replace dairy ingredients with plant ingredients. To the author's knowledge, no research deals with cereal based cheese analogues, and research is rather focused on soft cheese-like spreads based on soya and peanut.

Chavan & Jana (2007) reported inferior product properties of cheese analogues containing high levels of vegetable proteins instead of casein: adhesive/sticky consistency, lower hardness, stretchability and elasticity, and often poor flavour. Therefore, some studies use pastes, rather than milk-like beverages to overcome the texture problems; An imitation cheese spread obtained from a peanut paste resulted in a texture similar to commercial dairy-based products (Santos et al., 1989). Further, peanut was described as favourable due to its bland flavour and light colour (Chavan & Jana, 2007). Soybeans on the other hand are known to have an undesirable beany taste and to result in a grainy texture (Q. Li, Xia, Zhou, & Xie, 2013). In contrast to dairy products, plant-based products like sufu attained little scientific interest but the adaption of sufu processing technologies, shown in Figure 2, could be an interesting tool for other PBMSs.

Some research tried to incorporate other plant ingredients into traditional dairy products: Lupin-BMS was incorporated in tofu, which showed a neutral effect on texture, flavour and overall acceptability when replacing 40% of soya-BMS by lupin-BMS (Jayasena, Khu, & Nasar-Abbas, 2010). Lupin-BMS was also used in dairy based cheese and showed to enhance taste, texture, flavour, and overall acceptability of both fresh and matured cheese at concentrations of 25% (Elsamani, Habbani, Babiker, & Mohamed Ahmed, 2014). Sesame protein isolate showed to increase hardness, cohesiveness, adhesiveness, and gumminess in cheddar cheese since the sesame proteins contributed to the micro-structure via protein micelle cluster interaction in the curd (Lu, Schmitt, & Chen, 2010). Such research show promising opportunities for products since they are nutritional more valuable than the traditional products with a higher protein content, however the use of dairy ingredients excludes dairy-free diets followers. Nevertheless, these products followed the traditional cheese production steps, unlike most of the commercial dairy-free products, which are produced by blending ingredients simply together and incorporating different additives to achieve the desirable texture and taste (Chavan & Jana, 2007). Zulkurnain et al. (2008), for example, used tofu, and blended it with oil, salt, and 3 different polysaccharides, in order to achieve a similar texture to cream cheese: Carrageenan was used to impart firmness, maltodextrin provided body to the product, while pectin prevented syneresis but introduced viscous behaviour to the final product. Valuable insights about different production strategies for a soya based cheese spread are given by Li et al. (2013); the results showed that a combination of glucono-delta-lactone and LAB fermentation together with enzymatic hydrolysis resulted in the best spreadability, more acceptable sensory features, and a stable homogeneous structure.

Generally, plant-proteins possess very different molecular and functional properties compared to casein due to their larger molecular size and complex quaternary structure (Bachmann, 2001). As described in the section about yoghurt-like products, various technologies, like the application of proteases, acids, or heat treatments, need to be considered to improve the solubility, emulsification, and coagulability of those proteins. For example, the melting ability of imitation mozzarella cheese was improved by partial enzymatic hydrolysis of soya protein isolate by using a mixture of amylases and rennet (Nishiya, Tatsumi, Ido, Tamaki, & Hanawa, 1989). Not only proteins play a crucial role in the cheese matrix: fat determines the properties equally. It contributes to a creamy mouth feel, flavour, and texture. Fat globules occupy space in the protein matrix and prevent the formation of a dense network, which would result in a hard, corky product (Bachmann, 2001). Generally, the phase state of fat, i.e. solid or liquid, determines the texture of the product. The study of Lobato-Calleros et al. (1997) exhibited this clearly: Soybean fat imparted hardness and adhesiveness to cheese analogues, but decreased cohesiveness and springiness, while the opposite effect was observed for soybean oil and butterfat. The melting points of plant fats are generally low, around room temperature. To achieve the desired hardness in a cheese matrix, vegetable oils are often hydrogenated to increase the melting point. Since not only the physical, but also the chemical properties, play a crucial role, partial hydrogenation can help further to improve the texture: Saturated fatty acids promote the adsorption of lipophilic groups of the proteins onto the surface of fat globules, thus improving the emulsion and forming a reticulated matrix (Lobato-Calleros et al., 1997). However, this procedure reduces the nutritional value of plant fats drastically and increases the risk of cardiovascular and coronary heart diseases (Mozaffarian & Clarke, 2009). Moreover, the application of EPS producing strains could offer opportunities to improve the hardness, and structure of plant based cheese analogues, since this showed good results for fat reduced dairy cheeses (Awad, Hassan, & Muthukumarappan, 2005; Zhang et al., 2015).

Other related products

Cow's milk can be further formulated to other products and so can PBMSs; cream alternatives, ice cream, chocolate, and infant formulas~~, and pharmaceutical beverages~~ to name just a few. Although not much literature is available on these kinds of products, the ones available show promising results with new approaches. Certain processing steps are applied to achieve different products. Bastıoğlu et al. (2016) spray-dried a melon seed-BMS to obtain an instant powder. The product was obtained by a simple extraction, meaning crushing and blending of the melon seeds with water prior to a filtration. The spray dried powder prolonged the shelf life of the product and the prepared beverage maintained the quality characteristics of fresh melon seed-BMS. Another usage of dehydrated PBMS was studied by Aidoo et al. (2010): peanut-and cowpea-BMSs were drum dried and formulated into a fine powder with a hammer mill and used to prepare dark chocolate. Unfortunately, the recipe of the chocolate is not published, but the obtained chocolates showed good acceptability in a sensory test, with no significant differences to the control chocolate, prepared with skimmed milk powder. A tiger-nut BMS was used to replace cow’s milk in an ice-cream formulation, the results showed that even a complete replacement led to no significant differences in physical properties, like whipping, viscosity, overrun and meltdown, and sensory properties when compared to a dairy product. Additionally, the tiger-nut BMS enhanced the growth of the incorporated probiotic bacteria strains (El-Shenawy, Abd El-Azi, Elkholy, & Fouad, 2016).

Law, governmental and global interest about dairy substitutes

Product names like "milk" and "cheese" are traditional dairy terms and consumers relate such products essentially to be dairy-derived. However, PBMSs are labelled with the same descriptors to achieve familiar expectations quite often. The discussion from both sides (dairy, and non-dairy) whether this is misbranding and consumer deception, is not uniformly answered yet. The European commission regulates the term “milk” clearly, to be exclusively used for “the normal mammary secretion obtained from one or more milkings without either addition thereto or extraction therefrom” (Council of the European Union, 2007). Further designations related to dairy products are reserved also exclusively for milk products, i.e. whey, cream, butter, buttermilk, butteroil, caseins, anhydrous milkfat, cheese, yogurt, kephir, koumiss, viili/fil, smetana, fil (Council of the European Union, 2007). The European commission allows exceptions for traditional products (European Commission 2010). Such exceptions include products like “Leberkäse”, a meat product from Germany, “Crème d’anchois”, a fish spread from france, coconut milk, and “Leche de almendras”, almond milk from Spain. Therefore, coconut milk and almond milk are the only PBMSs allowed to be labelled as milk in the European Union. To the author’s knowledge, in no other country regulations like this are applied.

The idea of sustainable food production has become very important to local governments along with many food industries around the world. PROTEIN2FOOD is a project funded from the European

Union to improve human health, environmental sustainability, and biodiversity. The aim of this project is to increase the production and consumption of high value plant proteins. The project takes the whole product chain into account, from field to fork, to enhance the sustainability of their production and processing and to create innovative, high quality, protein-rich food products, including dairy-substitutes (PROTEIN2FOOD, 2015). The government of the United Kingdom recommends, in their recent publication "The Eatwell Guide", to replace dairy products with plant-based alternatives for a healthy diet (NHS England, 2016). Furthermore, "Protein Challenge 2040", which is a global coalition of stakeholders coming from various backgrounds, was founded to create a worldwide network of international businesses and non-governmental organisation to face the growing demand of proteins (Forum for the Future, 2016). These projects are just a few selected examples, but represent well the interest of different stakeholders. A key interest for all of them is to improve the public awareness, and knowledge regarding food and proteins. People's purchasing decision is highly related to cultural and social norms. Promoting plant-based food, and educating about health and sustainability can change this cultural and social environment.

Impact on climate and land use; Sustainability

Cereals are one of the most important energy sources in the world; they contribute to about 50% of the average daily energy intake in most populations, and in some developing countries they can contribute up to 70% of the energy intake (De Anton Migliorati et al., 2015). The world's population is increasing rapidly and is putting enormous pressure on food systems to provide enough food to feed the growing population. Food systems depend heavily on our natural resources like land, water, and energy resources like fossil fuels and natural gas, and increased pressure on these resources contributes massively to many of the environmental problems of today, such as greenhouse gas emissions and eutrophication (Jeswani, Burkinshaw, & Azapagic, 2015). The growth in food consumption per capita worldwide is also putting an increased pressure on the cereal production in the agricultural-food industry (Henry, Rangan, & Furtado, 2016). Even though land plants are an important source of energy in the human diet, relatively cheap and very abundant source of protein, the direct consumption of plant proteins is still relatively low (Day, 2013). There has been a move towards increased meat and dairy consumption in the last decade, which is especially evident in developed countries and consequently increasing the demand for cereals further as a main source of feed for the animals with the U.S. animal feed industry being the largest feed producer in the world producing over 120 million tonnes of animal feed in 2004 since the consumption of meats per capita in the U.S. in 2003 was 90.5 kg/year (Sapkota, Lefferts, McKenzie, & Walker, 2007). However, the conversion of plant proteins to animal proteins is very inefficient, since 75- 90% of the grain and consequently 65-90% of the plant protein fed to livestock is lost in conversion (Grigg, 1995). Meat and dairy production are responsible for a massive share in the food related environmental pressure, with the production of animal proteins requiring about 100 fold more water compared to the production of the same amount of plant based proteins (Day, 2013). Due to the constant rise of the global population, food security is becoming an increasing problem for the agricultural-food industry. Better use of plant based proteins is becoming more critical for sustainable food consumption, as the supply of animal based proteins reaches its maximum production capacity (Day, 2013). In this regard, PBDSs have the opportunity to expand their market and serve as a plant-based food, but especially, as a plant-based protein source in the diet.

However, the production of soya and almond, base for the most popular PBMSs, are facing already environmental problems; the rapidly expanding market for soya is causing a threat to the environment with a huge impact especially in South America. The expansion of soybean plantations into fragile ecosystems, like the rainforest, or savannahs, is contributing to climate change, and jeopardizing biodiversity and endangered species (Fearnside, 2001). A similar problem is faced by the almond agriculture: California is currently producing 80% of the world’s almonds (Sarah Theodore, 2015). Almonds have large water requirements, however California is suffering from its fifth year of severe drought (California Water Science Center, 2016). Therefore, alternatives need to be found. Plants, which are more resistant to climate changes, like peanuts, quinoa or peas for example, are already gaining in popularity (Sarah Theodore, 2015).

Nutritional properties

~~Nowadays, consumers are avoiding dairy products based on health concerns, like cholesterol and antibiotic residues in cow's milk.~~ ~~Further, it is widely known that dairy~~Dairy products negatively affect a great number of the world's population, as they cause adverse reactions within the body of the affected individual, including lactose intolerance and cow's milk allergy (Crittenden, Bennett 2005). At the same time, it needs to be noted that milk and dairy products are a fundamental source of nutrition for many people all around the world (FAO, 2013) and furthermore, beneficial effects associated with the consumption of these products have been shown in many studies (Mckinley, 2005; Michalski & Januel, 2006). However, ~~Nowadays,~~ consumers are avoiding dairy products based on health concerns, like cholesterol and antibiotic residues in cow's milk (Monitor, 2005)~~,~~. PBDSs are purchased for their health and wellness benefits (Mintel Group Ltd, 2016; Monitor, 2005). One major downside of PBMSs is the low protein content of most of the products available on the market. Half of the samples analysed by Jeske, Zannini, & Arendt (2016) contained less than 0.5% proteins and only samples based on soya reached values comparable to cow's milk with 3.7%. Even if for most of the population an overconsumption of protein is the case (Ranganathan, Vennard, Waite, & Dumas, 2016), if cow's milk is replaced by PBMSs, consumer awareness is still very important. Inappropriate use of PBMSs in the diet of young infants can lead to severe malnutrition. Le Louer et al. (2014) reported for several cases protein-calorie malnutrition and deficiencies of minerals and vitamins with severe consequences for the infants. In addition to the low protein quantity in some PBMSs, the quality of plant-based proteins can be inferior compared to cow's milk proteins, which has a protein digestibility-corrected amino acid score of 121. Plant proteins show lower values ranging from 91, 90 for soya and cashew, and down to 23 for almond (Ahrens, Venkatachalam, Mistry, Lapsley, & Sathe, 2005; Freitas, 2012; Schaafsma, 2000). However, the market is developing and products are changing: So Delicious Dairy Free released an almond-based beverage~~.~~, which claims to contain ~~with~~ 5 times more protein than ~~most of the~~ other almond milks available on the market. This product is ~~E~~enriched with pea and rice protein to ~~it~~ reach~~es~~ 2 g of protein per 100 mL of product (So Delicious Dairy Free 2016). Furthermore, fermentation has been shown to improve the protein digestibility (Holzapfel, 1997; Taylor & Taylor, 2002), likewise, the bioavailability of minerals and other micronutrients (Greffeuille et al., 2011; Watzke, 1998). Plants are a superior source for nutrients like vitamins, dietary fibres, antioxidants, and flavonoids, which have shown nutritious and health-promoting properties. For example, the level of plasma cholesterol and low-density lipoprotein cholesterol was decreased when cow's milk was replaced by an oat based beverage due to the ß-glucans in oats (Önning, Åkesson, Öste, & Lundquist, 1998). Also, fermented oat-BMS showed a lower postprandial glucose response than cow's milk yoghurt (Lindström et al., 2015). Again, in this study the ß-glucans were proposed to be responsible for this difference. The impact of EPS was studied as well but appeared to have no impact on the glycaemic response (Lindström et al., 2015). In general, the glycaemic index for several PBMS is moderate with values from 47 to 64. Rice- and coconut BMSs showed however high values, reaching values of 100 for the glycaemic index. Due to the low carbohydrate content of the coconut-BMS, this sample had a low glycaemic load (4.81), whereas the rice-BMSs showed values comparable to Coca-Cola (>16) (Jeske et al., 2016). Cow's milk is an important source for calcium, iodine, vitamin B12 and riboflavin. To overcome the risk of malnutrition most of the commercial PBMSs are fortified, but consumer awareness of this is very important. In this regard an interesting point is the potential renal acid load, which indicates the amount of acid produced during metabolism. The consumption of animal proteins increases the acid load in the body, whereas fruits and vegetables generally decrease it. The created acid needs to be neutralized. For this purpose, the body uses mainly calcium, which acts as a very effective base. ~~If the calcium is not supplied sufficiently by the diet, the calcium is taken from the bones, which makes them weaker and fractures are more common~~ (Barzel & Massey, 1998; Thorpe & Evans, 2011). To draw a line back to the calcium contents in cow's milk and PBMSs, it is not only important how much calcium is provided by the diet, but the way the entire food system is metabolised is essential. Some plants can contain anti nutritional compounds, like phytates and trypsin inhibitors, which impart the bioavailability of nutrients. By applying certain treatments these can be combated by fermentation, germination, chelating agents, or exogenous phytase (Buddrick, Jones, Cornell, & Small, 2014; Kumar, Sinha, Makkar, & Becker, 2010; Savelkoul, Van Der Poel, & Tamminga, 1992), or heat treatments (Friedman, 1996). Processing steps like heat treatments can destroy heat sensitive vitamins like B1, B$_6$, B$_{12}$, and D$_3$ and therefore, should be applied carefully. Further health benefits can be attained from probiotics used during fermentation. As aforementioned, probiotic strains grow successfully in PBDSs enhancing functional properties of the product. Probiotics are believed to suppress potentially harmful organism in the intestine, stimulate immune system, and prevent cancer (Kreisz et al., 2008). PBDSs and related products meet the increasing demand for healthy food and beverages and satisfy diets such as vegetarianism and veganism (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014). They are free of lactose and cow's milk proteins, but a lot of products are based on soya~~, oat~~ and peanut which are allergens also. Since 14% of the individuals who suffer from cow's milk allergy also have reactions against soya (Zeiger et al. 1999), other PBMSs should be considered to exclude more allergens and include more consumers. The avoidance of allergens is increasing and part of a healthy lifestyle for many people. ~~Since~~ An increasing number of consumers ~~of PBDSs are generally speaking very~~ seek consciously for products which are gluten-free, nut-free or even completely allergen-free~~about Gluten-free products~~ (Sarah Theodore, 2015).

Consumer acceptance and sensory characteristics

Consumers expect plant products, that resemble animal products, to be similar according to the law of similarity (Adise, Gavdanovich, & Zellner, 2015). However, a good approach from industry and consumers would be to appreciate the taste of the plant ingredients. Would a costumer not expect to taste quinoa in a quinoa-BMS, especially considering the high value and price of quinoa? Conflicting with this, simply the visual similarity of a plant-based animal product substitute and labelling it as such is increasing primarily the willingness of purchase, but if the sensory properties do not match the expectations, then the consumer might be disappointed and dislike the product even more (Zellner, Strickhouser, & Tornow, 2004). Also, an important point in this regard is a phenomenon called neophobia; Novelty of a product can evoke prejudices, simply because consumers are not familiar with the product. They expect the taste to be unpleasant and sometimes even assume the food to be dangerous (Adise et al., 2015). Dealing with these problems, the advertisement of such plant food alternatives is a key point. In an internet survey, 48% stated that they would purchase PBMSs because of its good taste (Mintel Group Ltd, 2016). However, sensory evaluations by ~~(Outi E.~~ Mäkinen et al., (2014), including lactose free bovine milk, soya-, oat-, quinoa- and rice-BMSs, showed hedonic ratings were the highest for bovine milk, and slightly lower likings were obtained for the PBMSs, besides the quinoa-based one, which was described as ‘‘dislike moderately’’. Furthermore, the panellists were asked about their future intention of consuming more PBMSs; 86% of the panellists stated the need to improve the taste of PBMS, and 73% would consume more if the products had proven health benefits. Proof of environmentally friendliness was considered to be an argument of purchase by 43% (~~Outi E.~~ Mäkinen et al., 2014). Since PBMSs are more or less suspensions of disintegrated plant material in water, the products can have a chalky mouth feel due to large insoluble particles (Durand, Franks, & Hosken, 2003), which can be removed by homogenisation or filtration. Many legume-BMSs are perceived as “beany” and “painty”, which occurs as a result of lipoxygenase activity, or possess of compounds with off-flavours. Quinoa, for example contains saponins, which cause bitterness and cause other disadvantages like decreased protein bioavailability (Pineli et al., 2015). By applying certain processing steps, like blanching, or soaking and maceration in acidified saline solution, enzymes can be easily inactivate (El-Shenawy et al., 2016; Yuan, Chang, Liu, & Xu, 2008), or undesired compounds can be removed (Pineli et al., 2015).

In several studies fermentation proved to be a useful tool to control texture, and especially, a desired flavour, as indicated earlier. *Streptococcus thermophilu~~e~~s* and *Lactobacillus bulgaricus* are commonly used as starter cultures in yoghurt production, which provide a huge range of flavour compounds; Lactic acid itself is one of the most important components contributing to yoghurt flavour. Together with formic, butanoic, and propanoic acids and especially the group of carbonyl compounds like acetaldehyde, acetone, acetoin, and diacetyl, it belongs to the most important aromatic components (Routray & Mishra, 2011). A screening of different strains applied to an oat based beverage produced by Mårtensson et al. (2000) resulted in low consumer acceptance if fermented with *Streptococcus thermophilu~~e~~s* and *Lactobacillus bulgaricus*, while samples fermented with *Leuconostoc mesenteroides* achieved pleasant flavours and a good taste in the oat-BMS. Several aroma compounds were identified in cereal and soya based yoghurt-like beverages which were fermented with different strains including *Lactobacillus plantarum*, *Lactobacillus rossiae*, *Weissella cibaria* and *Pediococcus pentosaceus*. Alcohols and aldehydes were the most abundant ones amongst other kind of aroma compounds. Products were not rated hedonically, but a characterization of sensory attributes was performed. Most of them were described as fruity, sour, and with cereal attributes but with little to no dairy notes (Coda, Lanera, Trani, Gobbetti, & Di Cagno, 2012). Mårtensson et al. (2002) did some further work on oat based yoghurt-like product fermented with *Pediococcus damnosus*. They succeeded to produce a product that could not be differentiated from a dairy equivalent by a sensory preference test.

Considering the cheese-like products, Ahmad et al. (2008) reviewed the flavour improvement of soya cheese and found that fermentation leads to better acceptability since cheese flavours are mainly produced by LAB with cultures like *Lactococcus lactis* ssp. *Lactis*, *Lactobacillus helveticus*, *Lactobacillus casei*, *Streptococcus lactis* ssp. *maltigenes* and *Lactococcus lactis* ssp. *cremoris*. However, the flavour in dairy cheese is a complex system of several compounds, and especially ripened cheese has an intense flavour resulting from the breakdown of casein proteins and milk fat into amino acids, amines, and fatty acids (Ahmad et al., 2008). Most studies deal with cream cheese analogues and to the author's knowledge no study exist exploring the sensory attributes of a ripened cheese analogue in detail. Soya-based products fermented by *Lactobacillus. casei* ssp. *rhamnosus* showed cream cheese compatible flavour and texture, with containing diacetyl as a flavour component (Hofmann & Marshall, 1985).

Future of plant-based dairy substitutes and thriving trends

The market of PBDSs is growing rapidly and is becoming more and more popular all over the world. Figure 3 summarizes the different influences and mechanisms that determine the variety of products available on the market. The European market is still in its infancy; however it is believed to grow during the next 5 years by nearly 50%. While soya is still the leading crop, other plants are trending and the variety of PBDSs is growing (Mintel Group Ltd, 2016). In the period of 2014-2015, more products based on other ingredients than soya were lunched. Consumers are opting for a variety of flavours and are getting more willing to experience new products. Soya has some disadvantages: first and foremost, it is among one of the most common food allergens. By using allergens, a big part of consumers is excluded. In total, a fifth of the population is affected by allergic diseases (including

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asthma), and more than a fifth of U.S. consumers is avoiding nut-/peanut-containing foods (Sarah Theodore, 2015). The avoidance of allergens is increasing, even for consumers who are not directly suffering from it. Therefore, the use of allergens will discourage more and more consumers from purchasing such products. Furthermore, soya beans are more than likely to be genetically modified: In the US, 94% of the soya varieties are genetically engineered (USDA.gov, 2016). Consumers are not well educated about this topic but 16% of PBDS-consumers are avoiding ingredients that are genetically modified (Mintel Group Ltd, 2016). Moreover, the environmental impact of soya and almonds is alarming as discussed in the chapter pertaining to climate impact. For all those reasons, the market is believed to change. This can be observed already in the "new" European market: rice/grain/nut/seed-based drinks were growing by 380% from 2012 to 2015, whereas soy-based drinks "just" doubled their market in Germany. New plants already arose in or from these markets recently, like lupine or tiger nut. Tiger nuts are not real nuts but tubers and traditionally used in Spanish Horchata. It gained popularity in the U.S., possibly since it is fitting into the meal plan of the trending paleo-diet (Sarah Theodore, 2015). Lupine is a new plant to be explored as an ingredient in PBDSs. It shows promising qualities due to its high protein, dietary fibre, and antioxidants contents (Sujak, Kotlarz, & Strobel, 2006). It is part of the European project PROTEIN2FOOD and is incorporated already in many products, including milk-, ice cream- and yoghurt-like foods by a German start-up company (Sarah Theodore, 2015). These new products are just examples, which are starting the new driving, innovative change of the market. Consumers are going to look out for more than just milk substitutes. They opt for healthy functional foods, which benefit there overall wellbeing. Indeed, the dairy-market is going to be more of a competing market for the non-dairy products, since most non-dairy milk drinkers also drink dairy milk (Mintel Group Ltd, 2016). Another trend, observed in the dairy industry may spill over to the non-dairy market also: Dairy products, like yoghurt are getting popular as a healthy option to conventional snacks (Bos, 2016). The demand for food is expected to grow by 70% until 2050 (from 2006 on). Due to urbanization, people change their diets to increased calorie and especially protein intake. While the expected protein consumption is believed to grow by 80% until 2050, the source of protein needs to change from being primary consumed from resource intensive foods, like meat and dairy products to plant-based proteins (Ranganathan et al., 2016).

Forty-two % of consumers are already consuming PBMSs as a source of protein and 30% would be encouraged to drink more PBMS if the products contain more protein (Mintel Group Ltd, 2016). Therefore, the protein content is supposed to be one of the driving factors for the development of new products being thriving on the market. On the other hand, the lack of protein in some of the non-dairy milks, like rice and almond could be a limiting factor for their future success.

Conclusion

The market for PBDSs is growing fast and the demand is increasing. The presented work showed clearly that the product quality needs to improve to meet the consumers' aspiration in future times. Sustainability and nutritional quality will need to be further improved if PBDSs are substituting dairy products commercially successful. Keeping up with the competitive request of the market research is facing the challenge: further studies on functional properties, stability, and sensory acceptance are needed while keeping the cost and environmental impact in mind.

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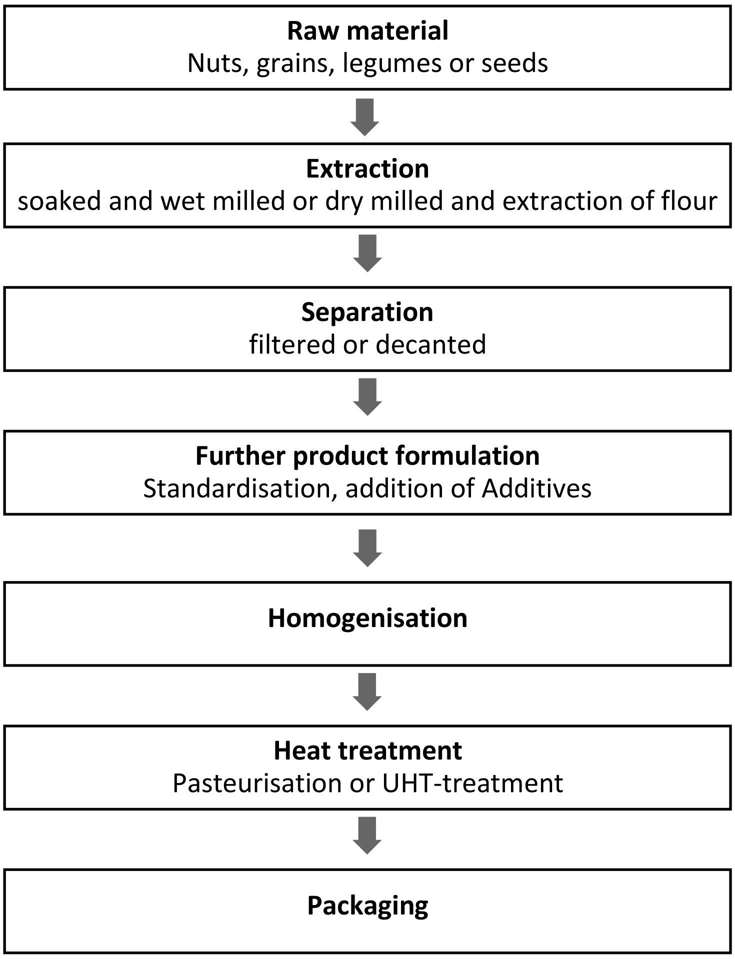
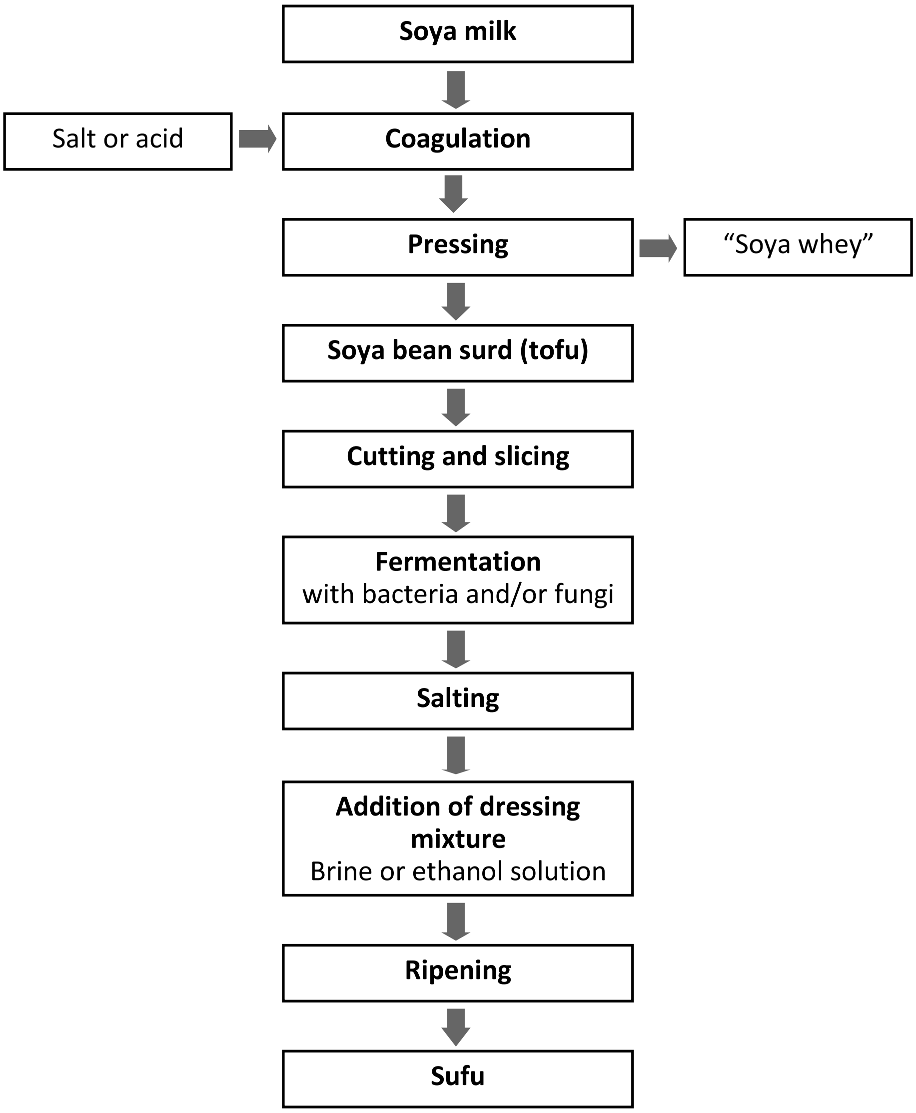
Figure 1



Raw material
Nuts, grains, legumes or seeds
Extraction
soaked and wet milled or dry milled and extraction of flour
Separation
filtered or decanted
Further product formulation
Standardisation, addition of Additives
Homogenisation
Heat treatment
Pasteurisation or UHT-treatment
Packaging

Figure 2



Soya milk
Salt or acid
Coagulation
Pressing
"Soya whey"
Soya bean surd (tofu)
Cutting and slicing
Fermentation
with bacteria and/or fungi
Salting
Addition of dressing mixture
Brine or ethanol solution
Ripening
Sufu

Figure 3

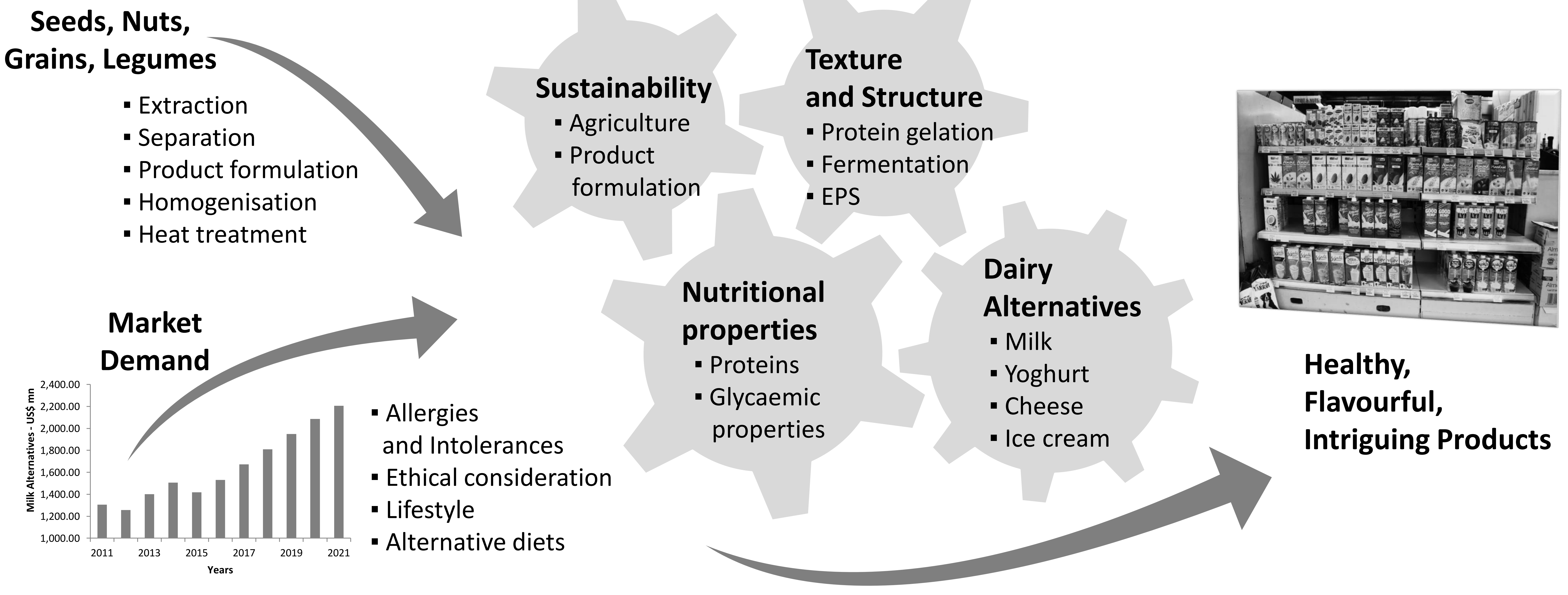

Seeds, Nuts, Grains, Legumes
▪ Extraction
▪ Separation
▪ Product formulation
▪ Homogenisation
▪ Heat treatment
Market Demand
Milk Alternatives - US$ mn
2,400.00
2,200.00
2,000.00
1,800.00
1,600.00
1,400.00
1,200.00
1,000.00
2011
2013
2015
2017
2019
2021
Years
▪ Allergies and Intolerances
▪ Ethical consideration
▪ Lifestyle
▪ Alternative diets
Sustainability
▪ Agriculture
▪ Product formulation
Texture and Structure
▪ Protein gelation
▪ Fermentation
▪ EPS
Nutritional properties
▪ Proteins
▪ Glycaemic properties
Dairy Alternatives
▪ Milk
▪ Yoghurt
▪ Cheese
▪ Ice cream
Healthy, Flavourful, Intriguing Products

Figure captions

Figure 1 General manufacturing steps for the production of PBMSs

Figure 2 Traditional process of “Sufu” (soya cheese)

Figure 3 Influences and mechanisms that determine the variety of PBDSs on the market

Graphical Abstract